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EXAMINER	
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17

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/753,892	YAKUBOV, LEONID A.	
	Examiner	Art Unit	
	Scott D. Priebe	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *25 April 2004 and 20 June 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-22,31 and 39-54* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-22,31 and 39-54* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

The amendment filed 25 April 2003 has been entered. Claims 1, 5, and 16-22 have been amended and new claims 39-54 have been added. Claims 32-38 have been cancelled, as per instruction on page 2, line 2. According to the supplemental response filed 20 June 2003, the declaration under 37 CFR 1.132 filed 25 April 2003 was not the version of the declaration that had been reviewed by the inventor. It has been placed in the file, but has not been considered. The corrected declaration filed 20 June 2003 has been considered.

Claim Objections

Claims 32-38, 43 and 48 are objected to because of the following informalities:

Claims 32-38 were cancelled (page 2, line 2, of the reply filed 25 April 2003). However, these claims are identified in the claim set as being "(withdrawn)" on page 6, which does not comply with revised 37 CFR 1.121. Claims 32-38 should be identified as -- (cancelled) – in the claim set.

Claims 43 and 48 recite "who does not cancer" in the third to last line of each claim. This phrase is grammatically incorrect; the phrase lacks a verb, e.g. -- have--.

Appropriate correction is required.

Claim Rejections - 35 USC § 101 & 112

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 48-54 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 48-54 are directed to a method of treating an individual who has a disease or disorder associated with exposure to mutagenic stimuli, etc. with polynucleotides from an individual "who does not [have] cancer". The reply indicates that support for these claims is found on page 15 of the specification. While page 15 supports the treatment of individuals exposed to mutagenic stimuli, it does not support using polynucleotides specifically from individuals who do not have cancer.

Claims 1-22 and 31 remain rejected and claims 39-42 are rejected under 35 U.S.C. 101 because the claimed invention is not supported by either a credible asserted utility or a well established utility.

Claims 1-22 and 31 remain and claims 39-42 are also rejected under 35 U.S.C. 112, first paragraph. Specifically, since the claimed invention is not supported by either a credible asserted utility or a well established utility for the reasons set forth below, one skilled in the art clearly would not know how to use the claimed invention.

Claim 1 has been amended to delete "recombine with the genomic DNA of the cell by homologous recombination." However, this amendment does not change the scope of the claim, since the claim still requires that the "non-mutated sequences or desirable alleles" of the

polynucleotide molecules replace the "mutated sequences or undesirable alleles" in the genomic DNA of the individual. The replacement of one allele with another allele in this context is homologous recombination, regardless of whether the claim explicitly identifies it as such. Consequently, the claims still require that the claimed method involve homologous recombination. New claims 43-54 have not been included in this rejection, since they do not require the method involve homologous recombination. The original grounds of rejection have been repeated below, followed by additional new grounds of rejection.

The invention is directed to a method for treating a genetic disease or disorder in an individual having a mutation associated with the disease or disorder by administering to that individual a collection of polynucleotides of 100-3000 nucleotides which collectively comprise a complete genome, presumably from an individual who does not have the genetic disease or disorder, wherein some of the polynucleotides contain a non-mutated sequence or desired allele. The claim recites that a polynucleotide which comprises the non-mutated or desired sequence will replace the mutated or undesired sequence. The specification presents no experimental results which validate the claimed method, and based on the prior art discussed below relating to gene targeting, the claimed method would not have been credible on its face to one of skill in this art.

The invention is based upon the untested hypothesis that circulating genomic DNA arising from apoptosis is used by cells as a guide or template to correct mutations via homologous recombination. It was known that RNA and DNA could be taken up by cells by a saturable, receptor-mediated endocytotic or pinocytotic pathway, with the nucleic acid being predominately located in the cytoplasm, not the nucleus (Bennett, Antisense Res. Dev. 3: 235-

241, 1993). Since the pathway is saturable, a cell would be able to take up only a limited amount of DNA. In one study using λ phage DNA, human leukocytes *in vitro* were found to bind approximately 3000 molecules. Of the internalized DNA, 80% was degraded to <12 base pairs. Labeled oligonucleotides administered intravenously or intraperitoneally to mice were found to be degraded and excreted in the urine (30%) or incorporated into kidney, liver and intestine in high molecular weight DNA. Bennett concluded that this pathway was a DNA salvage pathway.

Gene targeting has been studied as a method for gene therapy. Gene targeting involves the correction of a faulty gene by providing a DNA molecule that contains the desired sequence such that it can undergo homologous recombination with the faulty gene and correct the faulty sequence. This strategy differs from gene augmentation or supplementation therapy, which involves delivering a therapeutic gene to a target cell, adding an exogenous gene to the cellular genome rather than correcting a faulty endogenous gene. Yáñez et al. (Gene Ther. 5: 149-159, 1998) reviews the state of therapeutic gene targeting. Unlike the instant invention, prior art gene targeting involves administration to cells of a single polynucleotide molecule that is homologous to the target sequence and contains the desired sequence. Yáñez et al. teach that while gene targeting has been achieved in isolated cells, it has low efficiency and is impractical for *in vivo* use. Gene targeting experiments *in vitro* using a single dsDNA molecule typically yield only 10^{-5} to 10^{-7} targeted cells per cell exposed to DNA. In contrast, gene therapy by gene augmentation or supplementation using viral vectors can result in transfection of greater than 10% of target cells, i.e. it is 10000 to 1000000-fold more efficient at delivering a desired gene to a target cell. Also, for every targeted cell obtained *in vitro*, in which homologous recombination occurred, 30 to 40000 cells are obtained in which the DNA molecule is integrated randomly by illegitimate or

non-homologous recombination. As a result, the gene targeting method is far more mutagenic than corrective, i.e. for every cell having a corrected mutation (homologous recombination), 30 to 40000 cells will have insertion mutations. The efficiency of gene targeting is also affected by the length of homology present in the DNA molecule used, where efficiency increases exponentially up to about 14 kb of homology between targeting DNA and target DNA. The claimed method uses DNA molecules with substantially shorter length of homology. The efficiency also depends on the degree of sequence identity between the targeting DNA and the target sequence, with isogenic DNA having the highest efficiency. Yáñez et al. concluded that *in vivo* gene therapy by gene targeting "is not viable at present." See Yáñez at pages 149-150, 156. Riele et al. (Proc. Natl. Acad. Sci. USA, 89: 5128-5132, 1992) observed that using isogenic DNA for gene targeting in mouse ES cells was 20-fold more efficient than DNA from a different mouse strain; however, the use of isogenic DNA did not reduce the level of non-homologous recombination (Abstract; Table 1). With limited exceptions such as in laboratory animals, the instant invention would necessarily involve non-isogenic DNA, especially in humans, which in general are highly out-bred.

In a paper published after the instant invention was made, Porter (Molec. Ther. 3 (4): 423-424, 2001) indicates that the main reason that little work is being published in the area of therapeutic gene targeting is the low efficiency, especially in contrast to gene addition therapy with viral vectors, and that the target cells are rare in the body. These factors make gene correction "look like a lost cause." Efforts to improve the efficiency *in vitro* over electroporation by other delivery methods had so far failed to significantly improve the efficiency (page 423).

This publication is additional evidence that one of skill in the pertinent art would not have found the claimed invention credible at the time the prophetic invention was made.

As discussed above, DNA binding to cells is saturable. The instant invention involves fragmenting genomic DNA into molecules of between 100-3000 base pairs or nucleotides. Haploid mammalian genomes are approximately 5×10^9 base pairs. If reduced to molecules of 1000 base pairs, the fraction of molecules comprising a single specific base pair, e.g. a mutation site, would be 2×10^{-7} (i.e. $1000 \div 5 \times 10^9$), or one in 5,000,000 molecules. If a mammalian cell binds and takes up 3000 molecules, then the probability that a cell of a population would bind and take up a single molecule having the specific base pair corresponding to a targeted mutation would be 6×10^{-4} (i.e. $3000 \times (2 \times 10^{-7})$). As shown above with isolated DNA targeting constructs, only 10^{-5} to 10^{-7} targeted cells per cell exposed to DNA will have a corrected gene, despite the cells being exposed to a vast excess of the desired replacement sequence. If unfractionated genomic DNA fragments is to be used, as claimed, then the expected gene targeting frequency would be expected to be substantially lower, since DNA binding and uptake by individual cells is saturable (3000 molecules per cell) and the fraction of the genomic fragments that would target a given mutation is about one in 5,000,000 genomic fragments. Since the prior art just before the instant invention was made shows that one of skill in the art did not believe gene targeting was viable for *in vivo* therapy when a single DNA targeting construct was used, one would not have expected the instant method using unfractionated genomic DNA to be viable either, since it would be expected to be far less efficient than the method taught in the prior art.

The specification reports two experiments alleged to relate to the claimed method. The first involved transfecting human cancer cell lines with fragmented human sperm DNA. In two cell lines the treatment caused reduction in expression of erbB2 and cyclin D1, but no such change was observed in a third cell line. After a time, all cell lines ceased proliferating. In the second experiment, administration of fragmented genomic DNA from one strain of mouse was administered to a second strain of mouse after birth. The treatment allowed establishment of ectopic tumors in the DNA treated mice using a tumor cell line which arose in the first strain. Tumors could not be established in untreated mice of the second strain. While it may be attractive to speculate that these results are explained by homologous recombination between the DNA fragments and the cellular genome, no evidence is provided that homologous recombination-mediated genomic changes were in any way responsible for the results observed. Given the low frequency of gene targeting observed with isolated targeting constructs and the even lower expected frequency using unfractionated genomic fragments, it is unlikely that homologous recombination is responsible. It would be at least as likely to conclude that the known non-homologous recombination that predominates in gene targeting, i.e. random insertion of DNA fragments, is responsible for the observed effects since such insertions are mutations and have the potential to exert a phenotypic effect. Alternatively, the effects may have nothing to do with recombination, whether homologous or non-homologous. Gilchrest et al., (US 5,470,577, Ref. BG) showed that liposomal delivery of fragmented salmon sperm DNA to a murine melanoma cell line caused an increase in melanin production (col. 3-4). Gilchrest does not teach what mechanism is responsible for this effect, however, it is unlikely to be due to homologous recombination between the non-homologous salmon sperm DNA and the murine

cell's genomic DNA. Gilchrest speculates that the DNA fragments may trigger an SOS-like response in mammalian cells, similar to that of bacteria, leading to expression of certain genes (col. 1, lines 36-43). Neither of the experiments demonstrates that the source of the DNA mattered, for example would salmon sperm DNA have achieved the same results. Thus, while the disclosed experimental results are provocative, they do not support the invention which is being claimed which requires that phenotypic effects be due to correcting mutations or alleles by homologous recombination; nor do they provide evidence that the claimed invention has a utility that is credible to one skilled in this art.

In addition to the original grounds of rejection (repeated above), the following new grounds of rejection are added. The instant specification does not provide direct experimental evidence relating to the correction of inherited disease alleles or correction of mutations. Ledoux (Prog. Nucleic Acid Res. Mol. Biol. 4: 231-267, 1965) reported that attempts to modify phenotypic characteristics of a variety of animals by administration of allogenic, total genomic DNA were unsuccessful with one exception. While there had been one report of a success in ducks, the original authors of this report and others were unable to reproduce it in ducks, and others failed with other animals. (See Ledoux (1965) at pages 234-236.) In one study (Bearn et al., Exp. Cell. Res. 17 (3): 547-549, June 1959), genomic DNA of pigmented rats was administered to albino rats in two ways: intraperitoneal injection into neonate rats (3 hrs old) or intra-uterine injection into 5-day pregnant rats. No pigment changes were observed in the treated rats. In regard to these failures, Yoon et al. (Expt. Cell. Res. 34: 599-602, 1964) examined the fate of radiolabeled, homologous donor thymus DNA injected into mouse testes *in vivo*. An

average of only one in 10^4 gonadal cells took up the labeled DNA, almost exclusively into the meiotic nuclei. Based upon the results, it was concluded that the probability of incorporating a particular marker DNA into a recipient germ cell nucleus was less than the spontaneous mutation rate, and this was proposed as an explanation for the failure to observe genetic transformation in higher organisms *in vivo* (page 602). The method examined in the studies reported by Ledoux (1965) is essentially the same as that of the claimed subject matter, albeit used for a different purpose, and illustrates a direct test of whether the claimed invention could credibly be used to alter an undesired allele in cells of an individual suffering an inherited disease. Based upon these results, one of skill in the art would have ample reason to doubt the credibility of the claimed invention.

Karpfel et al. (Expt. Cell. Res. 32: 147-216, 1963) examined the effects of intraperitoneal injection of isogenic, homologous, and heterologous DNA into mice. When anaphase chromosomes from bone marrow cells were examined, the treatment was found to result in a frequency of chromosome abnormalities (i.e. chromosome rearrangements) above background. The frequency of abnormalities was highest with heterologous DNA, then homologous DNA then isogenic DNA isolated from organs other than spleen; DNA from spleen showed no increase in abnormalities. The results of Karpfel suggest that administration of DNA *in vivo*, even isogenic DNA, is mutagenic. This result is consistent with results obtained with cultured cells where it was found that random integration of donor DNA into mammalian chromosomes, i.e. mutagenesis, occurs at a much higher frequency than does homologous recombination between the donor DNA and recipient chromosomal DNA. This evidence raises the possibility

that the cytostatic effect of genomic DNA administered to cancer cells may be the result of mutations produced by random insertion of donor DNA fragments.

With respect to the treatment of individuals exposed to mutagenic stimuli such as ionizing radiation, the systemic delivery of exogenous donor DNA to mammals, primarily rats and mice, following exposure to radiation was known to provide a partial protective or restorative effect on the mammals, both in terms of speeding recovery and improving survivability. For example, Wilczok et al. (Int. J. Rad. Biol. Rel. Stud. Phys. Chem. Med. 9 (3): 201-211, 1965) examined a method for promoting survival of animals, specifically rats in this study, pre-exposed to lethal or near-lethal radiation doses. The method involved the intraperitoneal or intravenous administration to lethally-irradiated rats of heterologous (from calf thymus, Ehrlich ascites, salmon and herring sperm) or homologous (from rat liver or spleen) genomic DNA. In addition to varying the source of the DNA, the effect of DNA molecular weight on survival was assessed. The experiments were designed to address a controversy in this art as to whether the beneficial effects of systemically administered DNA depended upon the degree of homology between the donor DNA and the genomic DNA of the recipient. Some reports had indicated that only isologous or homologous donor DNA provided a protective effect; while others has noted no difference in protective effect between isologous, homologous and heterologous donor DNA. The results obtained (Wilczok, Table 1) showed that the source of the DNA had no effect on the protective or restorative effect, but the size of the donor DNA fragments had a significant effect (650,000 MW is approximately 1000 base pairs). Twice as many rats treated with heterologous calf-thymus DNA (heterologous) averaging 12 kb survived as did those treated with rat liver DNA (homologous) of 8.9 kb. Reducing the size or denaturing

the calf-thymus DNA dramatically reduced the survival (Table 2). Wilczok discloses that it was known that the efficiency of adsorption and incorporation of heterologous or homologous DNA by live cells *in vitro* was the same for both types of DNA and more efficient for large DNA (page 208). The reference also discloses that most DNA, immediately following intraperitoneal or intravenous administration *in vivo,* was localized in spleen, lymph nodes, bone marrow, individual lymphocytes and the crypts of the small intestine, largely taken up by lymphocytes (page 208). Ledoux et al. (U.S. Clearinghouse Fed. Sci. Tech. Inform. No. 715018, 1970) reports results in similar experiments on mice treated with bacterial or mouse genomic DNA, ranging in size from 3 to 7.5 kilobasepairs, following sub-lethal irradiation. Ledoux (Table III) also observed no difference in effect based on the source of the DNA, mouse vs. bacterial DNA. The equal effectiveness of heterologous, even bacterial DNA, and homologous DNA in protecting against lethal radiation damage in rats and mice strongly suggests that homologous recombination is not involved in whatever mechanism is responsible for the protective or restorative effect. If it were the primary mechanism, one would not have expected fish DNA and especially bacterial DNA to have any effect whatsoever. Thus, at least for the correction of mutations induced by radiation damage, it is not credible that homologous recombination is involved in the therapeutic effects observed with treatment with genomic DNA.

Pfeiffer (Toxicol. Lett. 96, 97: 119-129, 1998) discloses that double-strand breaks (DSBs) in chromosomal DNA are potentially lethal. Ionizing radiation and radiomimetic drugs, e.g. bleomycin, induce double-strand breaks in chromosomal DNA (page 119-120). DSBs lead to cell death (particularly if unrepaired), mutagenesis and chromosomal aberrations. In mammalian cells, the primary mechanism for repair of DSBs is a process, called non-homologous DNA end-

joining (NEJ), in which any two DSB-termini are joined (ligated) regardless of sequence or structural configuration. If two termini originate from different DSBs, the repair process can result in chromosomal rearrangements, e.g. translocations, large deletions. (See pages 119-120.) One possible explanation for the protective effect of administering DNA to irradiated mammals is that the DNA fragments, which have DSB termini, taken up by irradiated cells might be used by the NEJ repair system to bridge DSBs caused by the radiation. The predominance of NEJ in repair of DSBs may also explain why gene targeting results primarily in random integration of targeting constructs, rather than homologous recombination between the construct and its homologous sequence in a chromosome.

Taubes (Science 298: 2116-2120, 13 Dec. 2002) illustrates the level of skepticism by those in the field of gene correction when faced with results that are at odds with or remarkable when compared to results obtained by older gene targeting methods. Taubes summarizes the rise and fall of the use of chimeraplasts for replacement of one sequence with a homologous sequence. Chimeraplasts are double-stranded RNA-DNA oligonucleotides that comprise a short sequence of DNA that is homologous with a target sequence except for the nucleotide to be replaced. Initially, the reported efficiency of the method was as high as 50% in cultured mammalian cells. This report received media attention, since if true, chimeraplasty would revolutionize gene therapy. However, Taubes notes that many gene therapy researchers expressed skepticism about the claims because the results were remarkable (much higher frequency of correction) and were not backed up by "iron clad" data (page 2116, col. 3). In the years subsequent to the initial reports, few laboratories have reproduced results with chimeraplasts that exceeded the frequencies of gene correction observed with gene targeting

vectors relying on large fragments of homologous DNA. Most laboratories, including many with extensive experience in gene targeting and gene therapy, have failed to get it to work at all (page 2120). Unlike the case of chimeraplasty, where at least some evidence had been provided that gene correction had occurred, the specification provides no evidence of sequence replacement by homologous recombination between recipient chromosomes and genomic donor DNA. Taubes shows that strong and unequivocal evidence of the efficiency of the claimed method would be required before one of skill in this art would find it credible that inherited alleles or acquired mutations could be corrected *in vivo* with high enough efficiency to produce any benefit to the individual being treated.

Applicant's arguments filed 25 April 2003 have been fully considered but they are not persuasive. The rejection is based upon the lack of credibility that administration of fragmented genomic DNA from a healthy individual to an individual suffering a disease or condition resulting from an inherited mutation (e.g. cystic fibrosis, muscular dystrophy, hemophilia) or acquired mutation (exposure to mutagenic stimuli or cancer) would result in any therapeutic effect as the result of replacement of mutated sequences or undesirable alleles in the individual with non-mutated or desired alleles from the donor genomic DNA. The original grounds of rejection and new grounds of rejection set forth evidence from the prior art that one of skill in this art would not find the claim credible. The issue of specific or substantial utility was not raised in the rejection.

The rejected claims have been amended to remove explicit reference to "homologous recombination;" however as noted above, the replacement of one allele with another or of a

mutated sequence with a non-mutated sequence is homologous recombination. Claims 43-54 have not been included in this rejection because the claims do not require any particular mechanism of action, and at least for radiation exposure, it has been known for decades that treatment with DNA, homologous or heterologous, provides a restorative or protective effect though some unknown mechanism that is unlikely to involve homologous recombination, as discussed in the rejection.

Applicant takes issue with the relevance of Yanez et al., arguing that it is not relevant since the delivery of targeting DNA involved the use of vectors, while the claimed method does not. In response, first, Applicant is misconstruing what is meant by "vector" in this art, at least with respect to early targeting vectors. The heterologous portions of the vector are often the "mutation" that is to be introduced into the chromosome (Yanez, page 150, col. 1, full para. 1). The heterologous sequences are flanked by sequences that are homologous to the chromosomal target sequence. This is graphically illustrated in Deng et al. (Mol. Cell. Biol. 12 (8): 3365-3371, Aug. 1992), see Figs. 3 and 6. Second, the methodology discussed in Yanez represents attempts to increase the efficiency of gene targeting. As discussed above, methods essentially identical to those being claimed had been used in an attempt to genetically transform cells of intact animals with genomic DNA of phenotypically different strains of the animals without success, see Bearn, Ledoux (1965) and Yoon. Rather than using unfractionated genomic DNA, the methods described in Yanez use purified DNA constructs that all carry the desired allele or sequence. As pointed out above, for a haploid mammalian genome fragmented into 3000 nucleotide DNA molecules, only 1 in 5,000,000 molecules will carry the desired allele or sequence. Therefore, since the improvements described in Yanez were deemed insufficient to raise the efficiency of

gene targeting to a level that would make it useful for *in vivo* gene targeting, the claimed method would be expected to involve a less efficient method of gene targeting, and prior art attempts to produce phenotypic changes in animals by administration of homologous genomic DNA had failed, one of skill in this art would not have found the claimed method credible.

Applicant notes that Porter disclosed that gene targeting was just too attractive an idea to be abandoned, and that many labs are working on improving its efficiency, and that Porter is not directed to the same technology as the claimed invention. In response, the claimed invention is not directed to any such improvements in gene targeting constructs or delivery methods, but rather to the administration of fragmented genomic DNA, where only a very small fraction of molecules (about 1 in 5,000,000) would contain a corrective sequence. The simple fact that those in the art acknowledged that current technology using targeting constructs was inadequate, and that if gene targeting would ever be useful to treat inherited disorders, some as yet unknown technological improvements would be necessary, is evidence that the claimed method would not be seen as credible on its face by those in this art. Simply because a goal is attractive, does not make it possible to attain; take a perpetual motion machine, telepathy or cold fusion, for example. Applicant asserts that Porter concludes that gene targeting could be made to work. Applicant does not indicate where Porter draws this conclusion. It is unclear how Porter's statement that gene targeting, as a therapeutic treatment, looked "like a lost cause" can be construed as an indication that gene targeting could work.

Applicant argues that Bennet does not teach anything to indicate the claimed method would not work. However, Bennet was cited to show reasons, when taken with the other evidence of record, that the claimed invention would not be considered credible. Bennet shows

that most donor DNA molecules entering a cell would be degraded, and therefore unavailable to replace an undesired sequence by homologous recombination. Only one in 5,000,000 donor DNA molecules in the claimed method would carry the desired allele or sequence, and Bennet discloses that an individual cell would take in only about 3000 molecules. Therefore, the likelihood that a single cell would even take in a single donor DNA molecule carrying the desired allele is 1 in 1700. Of these cells, the single donor DNA molecule would be expected to be degraded in 80% of them. This further reduces the likelihood that a DNA molecule having the correct allele would even reach the nucleus and be available for homologous recombination to about 1 in 8500 cells. Yanez (Table 2) discloses that in cultured human primary cells, such as would be found in a human, the gene replacement frequency with a homogenous targeting construct is about 1 in 10,000,000 cells. When a homogeneous targeting construct is used, all molecules taken in would contain the correct molecule, and all molecules escaping degradation would contain the correct molecule. Combined with the evidence of Bennet, one would expect the use of genomic DNA in gene correction to be much lower, simply due to the reduced chance of a given cell taking in a correct molecule and the chance it would not be degraded after uptake.

Applicant argues that the statement by Yanez that gene targeting "is impractical for *in vivo* use," has no bearing on whether the claimed invention is enabled. However, Applicant is ignoring why Yanez makes this statement. The low efficiency of gene targeting in cultured cells, where all parameters can be controlled and optimized, strongly suggests that the efficiency *in vivo* on a per cell basis would be even lower. It is not an issue of whether the use of the claimed method would produce at least some cells *in vivo* where homologous recombination had occurred and an undesired allele (or mutation) had been replaced. The issue is whether the

extremely low efficiency of this process would allow gene correction in enough cells to have any detectable effect on the phenotype of the individual, and whether such a phenotypic effect would be the desired effect. The prior art of record shows that gene targeting constructs are far more likely to be inserted into chromosomes at non-homologous locations. Such insertions would not correct the undesired allele or mutation and would be mutagenic. At least some such mutations would be expected to be deleterious, which would perhaps partly explain the cytostatic effect on tumor cells.

Applicant argues that Reile is not relevant due to the amendment to claim 1. However, as explained above, this amendment does not remove the reliance of the method on homologous recombination. Furthermore, there is no doubt that treatment with heterologous or homologous DNA provides a protective effect from radiation damage. However, the evidence shows that this effect is unlikely to involve homologous recombination. As suggested by the Examiner above, one possible explanation for the effect is that the donor DNA fragments facilitate DSB repair, which even though mutagenic, allows the cell to survive the radiation-induced DSBs. Whatever the mechanism, this protective effect is non-specific, with respect to the source of DNA, and may not involve recombination at all, either homologous or illegitimate. However, the claimed invention is also directed to the correction of inherited mutations and mutations induced by chemical mutagens. Such correction could only be accomplished by homologous recombination, and would be inherent in the method whether the claim explicitly required it or not. Consequently, the teachings Reigle are directly relevant to embodiments of the invention requiring replacement of undesired sequences with desired sequences.

The Yabukov declaration under 37 CFR 1.132 filed 20 June 2003 is insufficient to overcome the rejection of claims 1-22 and 31 based upon a lack of credible utility as set forth in the last Office action. First, none of the experiments described provide unequivocal evidence that homologous recombination, i.e. replacement of a mutated sequence with a non-mutated sequence, was responsible for the effects described. With respect to para. 5, evidence has been provided that donor DNA fragments are far more likely to recombine with the chromosomal DNA by illegitimate recombination (random insertion), than by homologous recombination. The illegitimate recombination is itself mutagenic, and possibly the explanation for the cytostatic effects observed.

With respect to para. 6, first the experiment was carried out with cultured cells, literally bathed in DNA for an extended period of time. It is unclear how such conditions could be duplicated *in vivo*. Second, although the results shown are consistent with homologous recombinational repair of the caspase mutation in most of the cells, the magnitude of the result is suspect based upon similar experiments described in the gene targeting art. The PCR results are unconvincing since the fragments being monitored are considerably smaller (120 bp) than the size of the donor DNA fragments (200-3000 bp). A random insertion of the "wild type" sequence would be amplified as readily as would a sequence by homologous recombination. As disclosed in Yanez (page 150, bridging col. 1-2), PCR assays are not considered to be strong evidence, particularly, as here, where non-clonal cell populations are assayed. Also, Szybalska et al. (Proc. Natl. Acad Sci. USA 48: 2026-2034, 1962) describes a comparable experiment in which isogenic "wild-type" genomic DNA was used to transform human cells carrying a mutation in a gene encoding an enzyme. Under optimized transformation conditions, the maximum frequency of

transformation obtained was 1 in 2500 cells, at saturating donor DNA. The donor DNA was prepared by what was considered gentle techniques at the time to preserve "high specific transforming activity." The transformants were not analyzed at the molecular level to determine whether the gain of wild type gene function was due to correction of the mutation, as opposed to random integration of an intact wild-type gene. Considering the low frequency of transformation observed by Szybalska and the potential contribution to that total of random insertion, which was later shown to be more efficient than homologous recombination, more rigorous evidence is needed to show that the results described in para. 6 are due to homologous recombination. One possible explanation is that the cell culture was contaminated at some point with cells of a different cell line that could reproduce more frequently and were sensitive to TNF-alpha.

The experiments described in para. 7 are similar to those described above by Wilczok and Ledoux (1970), which disclose results that strongly suggest that the effect is not due to homologous recombination, since heterologous DNA is just as effective. With respect to para. 8, cyclophosphamide is a radiomimetic drug in the sense that the primary genotoxic and mutagenic effects ultimately involve double-strand breaks (see Anderson et al., Mutat. Res. 330: 115-181, 1995, at pages 123-125 and 138). Consequently, the observed protective effect by the DNA may share a similar mechanism with that responsible for the restorative effect that DNA treatment provides following radiation exposure.

With respect to para. 9-11, there is no evidence that the treatment with the DNA (Panagen) was responsible for any of the results observed with this patient. More importantly, there is no evidence that even if the DNA contributed to the results, that homologous recombination was involved.

Claim 48-52 and 54 rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for treating an individual exposed to ionizing radiation, does not reasonably provide enablement for treating individuals exposed to other mutagenic stimuli. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

The claims are directed to a method for treating an individual having a disease or disorder associated with exposure to a mutagenic stimulus, such as ionizing radiation (claim 53) or a chemical mutagen (claim 54). The method involves comprises administering to the individual complete genomic DNA obtained from an individual that does not have cancer. The claims do not require that the source of the donor DNA be from the same species as the recipient. However, the specification teaches primarily that the donor of the DNA and the recipient should be of the same species, in some instances the DNA is from the recipient prior to exposure to the mutagenic stimulus. The specification indicates the invention is based upon the untested hypothesis that circulating genomic DNA arising from apoptosis is used by cells as a guide or template to correct mutations via homologous recombination. The specification provides no working examples of the claimed invention.

As described under the preceding rejection, it was known in the prior art that systemic delivery of exogenous genomic DNA to mammals immediately following an otherwise lethal exposure to ionizing radiation could provide a partial protective or restorative effect to the mammals in terms of speeding recovery and improving survival. However, the effect did not depend on the source of the donor DNA. The effect did not depend on the homology between the

donor DNA and the recipient genomic DNA; completely non-homologous DNA was as effective as homologous DNA. See for example, Wilczok et al. (Int. J. Rad. Biol. Rel. Stud. Phys. Chem. Med. 9 (3): 201-211, 1965) and Ledoux et al. (U.S. Clearinghouse Fed. Sci. Tech. Inform. No. 715018, 1970). Consequently, it is highly unlikely that homologous recombination was responsible for the effect. The mechanism by which the DNA produces the protective or restorative effect appears to be unknown, i.e. there is no evidence of record that the mechanism was ever elucidated. There is no evidence of record that the treatment conferred any protection against the mutagenic effects of ionizing radiation.

Also, as described under the preceding rejection, the prior art disclosed that attempts to modify the phenotypic characteristics of different vertebrates by administration of homologous DNA failed, except for one reported success that could not be reproduced. See for example Ledoux (Prog. Nucleic Acid Res. Mol. Biol. 4: 231-267, 1965), Bearn et al. (Exp. Cell. Res. 17 (3): 547-549, June 1959), and Yoon et al. (Expt. Cell. Res. 34: 599-602, 1964). Subsequent work on cultured cells, showed that recombination in mammalian cells between the cellular genome and homologous donor DNA is an extremely inefficient process, and by far the predominant recombination is non-homologous or illegitimate recombination. See for example, Yáñez et al. (Gene Ther. 5: 149-159, 1998), Porter (Molec. Ther. 3 (4): 423-424, 2001), and Szybalska et al. (Proc. Natl. Acad Sci. USA 48: 2026-2034, 1962).

Taken all together, the administration of genomic donor DNA to an individual is unlikely to result in any meaningful level of homologous recombination between the donor DNA and the recipient genome, regardless of whether the individual was exposed to a mutagenic stimulus or not. Consequently, if any embodiment of the claimed method were to be operable for treating an

individual exposed to a chemical mutagen, it is unlikely that the donor DNA would act by correcting mutations produced by the mutagen. In fact, the prior art discloses that administration of the donor DNA it self would be mutagenic due to illegitimate recombination, i.e. the DNA is a chemical mutagen. See preceding rejection.

The specification discloses no other potential mechanism as to how administration of homologous DNA might provide a therapeutic benefit to an individual exposed to a mutagenic stimulus. There is no evidence of record that administration of genomic DNA, whether homologous or heterologous, to individuals exposed to chemical mutagens was known in the prior art to have any beneficial effect.

While it is not necessary for the application to disclose the mechanism by which an invention works, without such knowledge one of skill in the art cannot make predictions from the known effects of donor DNA in treating lethal irradiation as to whether such treatment would provide any benefit to an individual exposed to a chemical mutagen. The specification provides no working example that the claimed invention would provide any benefit whatsoever to an individual exposed to a chemical mutagen, i.e. there is no evidence that such embodiments are operable, and no reason to believe they would be. Evidence from the prior art discussed above indicates that even if a beneficial effect would result, it is unlikely that the beneficial result would be the correction of mutations. Consequently, one would be unable to predict what the beneficial result would be, and the specification provides no such teachings.

It has long been recognized in the chemical arts that the unpredictability of a particular art area may alone provide a reasonable doubt as to the accuracy of a broad statement made in support of the enablement of a claim. *Ex parte Singh*, 17 USPQ2d 1714, 1715 (BPAI 1991) ; *In*

re Marzocchi, 169 USPQ 367, 369-370 (CCPA 1971). As set forth in *In re Fisher*, 166 USPQ 18, 24 (CCPA 1970), compliance with 35 USC 112, first paragraph requires:

> that scope of claims must bear a reasonable correlation to scope of enablement provided by specification to persons of ordinary skill in the art; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws; in cases involving unpredictable factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with degree of unpredictability of factors involved.

Though not controlling, the lack of working examples, is, nevertheless, a factor to be considered in a case involving both physiological activity and an undeveloped art. When a patent applicant chooses to forego exemplification and bases utility on broad terminology and general allegations, he runs the risk that unless one with ordinary skill in the art would accept the allegations as obviously valid and correct, the examiner may, properly, ask for evidence to substantiate them - *Ex parte Sudilovsky*, 21 USPQ2d 1702, 1705 (BPAI 1991); *In re Novak*, 134 USPA 335 (CCPA 1962); *In re Fouche*, 169 USPQ 429 (CCPA 1971). A patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion. Tossing out the germ of an idea does not constitute an enabling disclosure. While every aspect of a generic claim need not have been carried out by an inventor, or exemplified in the specification, reasonable detail must be provided in order to enable the skilled artisan to understand and carry out the invention. *Genentech Inc. v. Novo Nordisk A/S*, 42 USPQ2d 1001, 1005 (Fed. Cir. 1997).

Consequently, due to the lack of credibility for the proposed mechanism of action of the claimed invention and lack of any other guidance either in the specification or prior art that would allow one to predict the effects of treating individuals exposed to chemical mutagens with

homologous or heterologous genomic DNA, the lack of any relevant working example, and the unpredictability of whether any embodiments involving chemical mutagen exposure are even operable, which if any are operable, and what benefit would be obtained, undue experimentation would have been required to practice the invention commensurate in scope with the claims.

The Yabukov declaration under 37 CFR 1.132 filed 20 June 2003 is insufficient to overcome this rejection. Para. 8 of the declaration describes an experiment where mouse or human genomic DNA was administered to mice suffering from leukopenia induced by cyclophosphamide. Administration of mouse genomic DNA, but not human genomic DNA, appeared to result in increased recovery from the leukopenia. These results do not show whether the beneficial effect was the result of recombination between the donor DNA and the genomic DNA of the mouse's cells, or that the effect was due to the correction of mutations, or any other mechanism. As disclosed in Anderson et al. (Mutat. Res. 330: 115-181, 1995, at pages 123-125 and 139, col. 1), the cytotoxic effects of cyclophosphamide are thought to be due to the blockage of DNA replication caused by interstrand cross-linking, not the induction of mutations. It is not clear from this experiment what effect of cyclophosphamide treatment was reduced or overcome by treatment with the genomic DNA. For the reasons set forth above, it is not likely to have corrected mutations induced by cyclophosphamide, so the protective effect is likely due to some unknown mechanism. Different chemical mutagens act by different mechanisms, and produce different types of mutations and different effects, not just mutations as illustrated by cyclophosphamide. Consequently, there is no way to predict whether the effect produced by the DNA following cyclophosphamide exposure would extend to other types of mutagen. This

particular embodiment is not described in the specification, and is not commensurate in scope with the claims with respect to chemical mutagens.

Claim Rejections - 35 USC § 102 & 103

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 43-53 are rejected under 35 U.S.C. 102(b) as being anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Sekiguchi et al., US 3,803,116.

Sekiguchi et al. teaches a method for treating cancer patients, e.g. humans, being treated with ionizing radiation that involves administration of genomic DNA isolated from individuals, e.g. from fish sperm or mammalian organs, alone or complexed with polyamines or polyamino acids. The DNA is reduced in size to the range of 200,000 to 500,000 MW, which is approximately 300-800 base pairs in length. The goal of the treatment is to reduce the deleterious side effects of the radiation treatment, e.g. leucopoenia. In a working example (col. 3-4), lethally-irradiated mice were treated with free herring sperm DNA or herring sperm DNA complexed

with spermidine. The treatment was effective to extend the average lifespan of the irradiated mice, and the fraction surviving past 30 days. In this case, lethality is a condition associated with the mutagenic stimuli.

The claims do not specify that the individuals having cancer or exposed to mutagenic stimuli be of the same species as the individuals from which the "polynucleotide molecules" are isolated. Sekiguchi does not explicitly teach that the donor DNA was isolated from individuals that do not have cancer, however fish sperm would not be cancer cells even if the fish had cancer, and commercially prepared DNA from mammals would likely include DNA prepared from individual mammals that did not have cancer (as opposed to being prepared solely from individuals having cancer).

Claims 48-53 are rejected under 35 U.S.C. 102(b) as being anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Ledoux et al. (U.S. Clearinghouse Fed. Sci. Tech. Inform. No. 715018, 1970).

Ledoux discloses treating irradiated mice by intraperitoneal injection of genomic DNA isolated from mouse intestine, of about 2 Mdal (approximately 3000 nucleotides) in length. The treatment improved the survival of the irradiated mice. In so much as DNA isolated from cancerous tissue was used in different experiments, it is presumed that the intestine DNA was not intentionally taken from mice with cancer.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Scott D. Priebe whose telephone number is (571) 272-0733. The examiner can normally be reached on M-F, 8:00-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Amy J. Nelson can be reached on (571) 272-0804. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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